Compound

This invention relates to compounds useful as contrast agents in magnetic resonance imaging and to methods of imaging using such compounds.

Magnetic resonance (MR) imaging is a well established imaging modality in which the image is derived from the intensity of the nmr signal from protons (usually water protons) in the subject under study. Because most tissue has an approximately 80% water content, contrast in MR imaging is attained by the application of pulse sequences that reveal differences in the relaxation times (T_1 and T_2) of the tissues. As with other diagnostic imaging modalities such as CT and ultrasound, contrast agents may be used in MR imaging procedures to enhance contrast in the images produced, e.g. to allow clearer differentiation between different tissue types or between healthy and non-healthy tissue. In MR imaging, the contrast agents conventionally are chelated paramagnetic species (e.g. Gd DTPA, Gd.DTPA-BMA and Gd HP-DO3A, available commercially under the trade names Magnevist, Omniscan and Pro-Hance), which achieve contrast enhancement because of their relaxivities, their ability to decrease the relaxation times of water protons.

A proposal has been made, in WO96/38184, that "triggered" paramagnetic metal ion complexes be used as MR contrast agents. As described in WO96/38184, the trigger mechanism has the paramagnetic complex being "turned on" as an MR contrast agent by the presence of a target substance which interacts with the agent complexing the paramagnetic metal ion so as to free an inner sphere coordination site and allow water molecule exchange to take place at the freed-up site. In the absence of the target substance, the complexed paramagnetic metal ion has no inner sphere coordination

sites available for water molecule exchange and in this state the contrast agent is considered to be turned off.

This concept of a triggered MR contrast agent however has a major defect which will hinder practical application of the concept. Thus in the "turned off" state the complex will still function fairly effectively as an MR contrast agent since both inner-sphere and outer-sphere water coordination contributes to the agent's relaxivity. The inventors of WO96/38184 indirectly acknowledge this drawback when they refer to the degree of change in MR signal that is sufficient to be detectable in the image as being as low as 2 to 5%, well below the conventionally accepted threshold of 10% (see for example Chem. Rev. 87: 901-927 (1987)). The relaxivity of the gadolinium chelates of WO96/38184 will be reduced by about one half (but not eliminated) if inner sphere coordination of water is prevented. Thus the triggered agents of WO96/38184 are not so much switched off as dimmed by about half by the absence of the target substance. Accordingly the selectivity and sensitivity desired by the authors is not possible due to the unavoidable outer-sphere contribution.

It has since been proposed by the applicants in WO98/47539 that triggered MR imaging of contrast agents may be achieved significantly more efficiently by using the "target substance" to change the contrast agent between states in which the relaxivity (r_1) differs by a factor of at least 5. This is achieved either by switching to a lower relaxivity state with little or no relaxivity or alternatively by switching on/off an inner sphere deriving relaxivity which is significantly higher than (e.g. 5 times or greater than) the outer sphere deriving component of the relaxivity.

Certain contrast agents which have now been found to be particularly suitable for use in "triggered" MR imaging techniques are those comprising lanthanide compounds which can be switched between first and second

oxidation states differing in relaxivity by a factor of 5 or more, preferably 10 or more, but can be much higher, e.g. at least 20, at least 100 or even significantly larger if the relaxivity of the low relaxivity state approaches zero. "Triggered" MR imaging is achieved using such agents as a result of a redox reaction.

Thus viewed from one aspect the invention provides a method of generating a contrast enhanced image of a human or non-human (preferably mammalian) animal subject which comprises administering to said subject an effective amount of a magnetic resonance imaging contrast agent and generating an image of at least part of said subject containing said agent, wherein said agent comprises a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, preferably at least 10, but can be much higher, e.g. at least 20, at least 100 or even significantly larger if the relaxivity of the low relaxivity state approaches zero, and which is convertible *in vivo* from said first to said second oxidation state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

In the method of the invention the change between high and low relaxivity states is effected as a change in the oxidation state of the lanthanide metal in the contrast agent between higher and lower relaxivity states. In this regard, the means for effecting the change between higher and lower relaxivity states may be localised normal or abnormal biological activity, an administered chemical agent or an applied physical means (e.g. illumination with light).

The change in oxidation state may give rise to a change in relaxivity in a number of ways, e.g. as a result of a change from a paramagnetic to a diamagnetic

state, from a diamagnetic to a paramagnetic state, or from one paramagnetic state to another. Conveniently, the change in relaxivity of the contrast agent is effected as a change from one paramagnetic state to another, e.g. from a non-spherically symmetric electronic ground state to a spherically symmetric electronic ground state, or a change from a non-spherically symmetric electronic ground state to a spherically symmetric excited state. The non-spherically symmetric state will have a much lower associated relaxivity than the spherically symmetric state and accordingly the contrast difference between the "on" and "off" states of the switchable agent is large.

Preferably, the contrast agent for use in the method of the invention is a chelate complex of a lanthanide metal ion in which the chelated metal ion is capable of redox conversion from one oxidation state to another (one or both of which are paramagnetic). On/off switching by a redox reaction may occur either as a result of oxidation or reduction of the chelated metal ion. Depending on the particular lanthanide metal present, its initial oxidation state and the nature of the complexing agent, this may bring about either a decrease or increase in relaxivity of the contrast agent.

Preferred contrast agents for use in the invention are those in which the "on position" corresponds to a state in which the relaxivity is as high as possible and in which the "off position" corresponds to a state in which the relaxivity is as low as possible, preferably close to zero. In this regard, contrast agents comprising Europium compounds, in particular chelate complexes of Europium, which are activated by switching between the II and III oxidation states, e.g. by biological activity or by redox reagents are particularly preferred for use in the method of the

invention.

Due to a half filled 4f shell, Eu(II) complexes have a spherically-symmetric electronic ground state ($^8S_{7/2}$) and therefore have long electron spin relaxation times and particularly high relaxivities. Eu(III) complexes, on the other hand, have a $^7F_0$ electronic ground state and very short electronic relaxation times Eu(III) is only paramagnetic because excited states must be considered, but these states are not spherically symmetric. Consequently, electronic relaxation times are very short and relaxivities are essentially zero. Oxidation of Eu(II) to Eu(III) thus causes a substantial loss of relaxivity which is readily detectable as a marked change in MR signal intensity. The transition from Eu(II) to Eu(III) thus provides a highly sensitive "on-off" switch. Moreover, the transition from Eu(II) to Eu(III) is particularly sensitive to oxygen concentration and pH:

$$Eu(II) + H^+ + \tfrac{1}{4}O_2 = Eu(III) + \tfrac{1}{2}H_2O \quad (I)$$

$$Eu(II) + H^+ = Eu(III) + \tfrac{1}{2}H_2 \quad (II)$$

Equation (I) is dominant when oxygen is present.

Suitable complexing agents for use in the invention are those which present the lanthanide metal, in particular Europium, in a biotolerable form, e.g. a polyaminopolyacid chelating agent of the type well known for MR agents and radiopharmaceuticals, for example DTPA, EDTA, DTPA-BMA, DO3A, DOTA, HP-DO3A, TMT, DPDP, etc. In this regard the reader is referred to the patent publications of metal chelates from Schering, Nycomed, Salutar, Bracco, Mallinckrodt, Guerbet, Sterling Winthrop, etc. Examples include US-A-4647447, US-A-5362475, US-A-5534241, US-A-5358704, US-A-5198208, US-A-4963344, EP-A-230893, EP-A-130934, EP-A-606683, EP-A-438206, EP-A-434345, WO 97/00087, WO 96/40274, WO 96/30377, WO 96/28420, WO 96/16678, WO 96/11023,

WO 95/32741, WO 95/27705, WO 95/26754, WO 95/28967, WO 95/28392, WO 95/24225, WO 95/17920, WO 95/15319, WO 95/09848, WO 94/27644, WO 94/22368, WO 94/08624, WO 93/16375, WO 93/06868, WO 92/11232, WO 92/09884, WO 92/08707, WO 91/15467, WO 91/10669, WO 91/10645, WO 91/07191, WO 91/05762, WO 90/12050, WO 90/03804, WO 89/00052, WO 89/00557, WO 88/01178, WO 86/02841 and WO 86/02005.

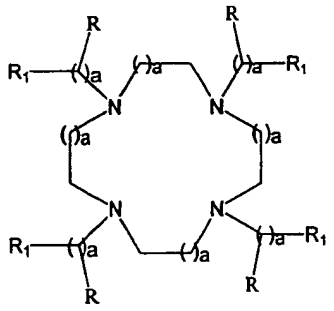
Thus appropriate complexing agents include macrocyclic chelants having an open coordination site for water, e.g. porphyrin-like molecules and the pentaaza macrocyclic ligands of Zhang et al (Inorg. Chem. 37(5):956-963, 1998), phthalocyanines, crown ethers e.g. nitrogen crown ethers such as the sepulchrates, cryptates etc., hemin (protoporphyrin IX chloride) and heme (available from Porphyrin Products, Inc. of Logan, Utah, USA) and chelants having a square-planar symmetry. Alternatively, the complexing agent may comprise a polyacid ligand capable of protonating a coordinating group thereby freeing up a coordination site for water molecules at a particular pH.

Other complexing agents of use according to the invention include polyoxadiazamacrobicyclic ligands ("cryptands") known to form stable coordination compounds ("cryptates") with several lanthanide metal ions, in particular with Europium (see J. Am Chem. Soc. 102(7): 2278-2285, 1980). In this regard, the (2.2.1), (2.2.2) and (2_B.2.1) cryptands are particularly suitable for use in the invention [the numerals within the parentheses refer to the number of oxygen atoms in the polyether bridges joining the nitrogen bridgeheads in the bicyclic molecule. Thus, (2.2.1) cryptand = 4,7,13,16,21-pentaoxa-1,10-diazabicyclo[8.8.5]tricosane and (2.2.2) cryptand = 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo [8.8.8]hexacosane. The ligand (2_B.2.1) is similar to (2.2.1) except that one of the central dioxyethylene groups is replaced by the analogous

catechol].

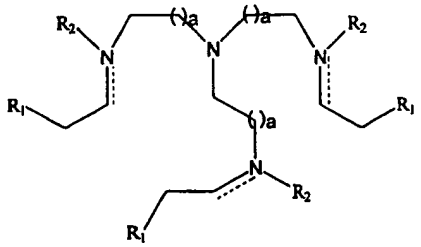
Particular Europium compounds for use in the invention include the following cryptates: $Eu^{II}(2.2.1)$, $Eu^{II}(2_{\beta}.2.1)$, $Eu^{II}(2.2.2)$ and the corresponding Eu^{III} complexes, $Eu^{III}(2.2.1)$, $Eu^{III}(2_{\beta}.2.1)$ and $Eu^{III}(2.2.2)$.

Suitable complexing agents also include ligands of formula (I)



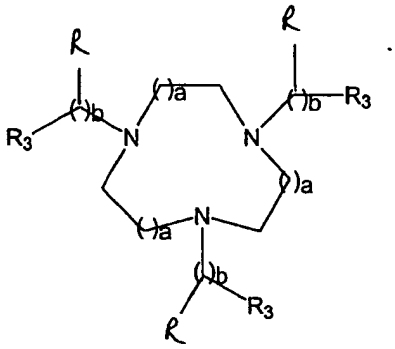
where each a independently represents an integer between 1 and 3, preferably 1, each R independently represents hydrogen or hydroxy and each R_1 independently represents a carboxylate, phosphate, thioacid, thiol, amino alkoxide or hydroxy group, preferably carboxylate;

formula (II)



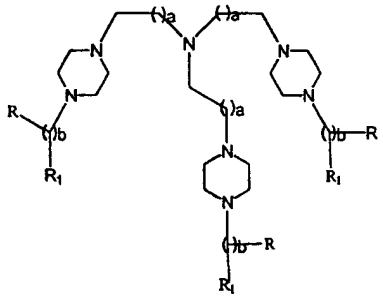
where a and R_1 are as hereinbefore defined and each R_2 independently represents hydrogen, C_{1-6} alkyl, e.g. methyl or isopropyl, aryl, e.g. phenyl, with the proviso that R_2 is absent when the double bond is present on the same nitrogen;

formula (III)

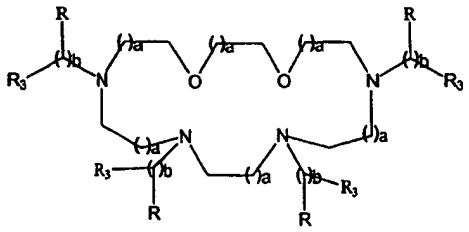


where a, R and R_2 are as hereinbefore defined, b is an integer between 0-3 and each R_3 independently represents R_1, $NR-NR_2-COO^{\ominus}$, or $N=N-COO^{\ominus}$ when b is positive or each R_3 independently represents $N=CH-COO^{\ominus}$ or $NR_2-CH_2-COO^{\ominus}$;

formula (IV)



where a, b, R and R_1 are as hereinbefore defined; and formula (V)



where a, b, R and R_3 are as hereinbefore defined.

Also of use are complexing agents of formula (VI)

$$\begin{array}{c} Y^1\text{-}L^1\text{-}A\text{-}L^2\text{-}Y^2 \\ | \\ L^3 \\ | \\ Y^3 \end{array}$$

where A is N, CR_4, P, P=O, *cis,cis,cis*-1,3,5-trisubstituted-cyclohexane or an N,N',N"-trisubstituted-triaza 9 to 14 membered macrocyclic ring;

L^1,L^2,L^3 are linker groups which are independently chosen from C_{1-4} alkylene, C_{4-8} cycloalkylene or C_{4-8} o-arylene;

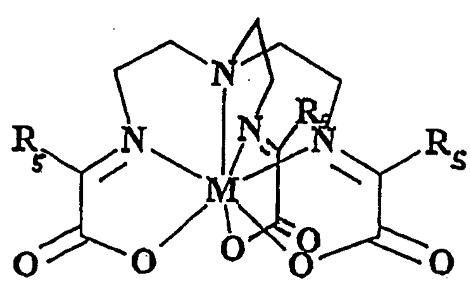
Y^1,Y^2,Y^3 are independently chosen from $-NH_2$, -B(=O)OZ, $-N=CR_5-B(=O)OZ$, $-NR_5-CR_6-B(=O)OZ$, $-N[CR_6-B(=O)Q]_2$ and $-O-CR_6-B(=O)OZ$ where B is C or PR_6, each Q is independently -OZ or $-NR_6$, and Z is H or a counter-ion;

each R_4 and R_5 group is independently chosen from H, C_{1-5} alkyl, C_{1-5} alkoxyalkyl, C_{1-5} hydroxyalkyl, C_{1-5} aminoalkyl, C_{5-10} aryl or C_{1-6} fluoroalkyl;

R_6 is OH, C_{1-6} alkyl, C_{1-6} alkoxyalkyl, C_{1-6} fluoroalkyl, C_{1-10} alkoxy or C_{5-10} aryl;

with the proviso that at least one of Y^1, Y^2 and Y^3 is $-N=CR_5-B(=O)OZ$.

For example



Specific complexing agents of use according to the invention include